

TABLE I.  
Effect of p-Arsenobenzamide and its Dithioglycollate on Viral Respiratory Infections in Mice.

| Virus          | Compound | Daily dose, mg | No. of mice | Lesion score |           | Lung weight |           |
|----------------|----------|----------------|-------------|--------------|-----------|-------------|-----------|
|                |          |                |             | T/C*         | % change† | T/C*        | % change† |
| Cat pn.        | I        | 0.4            | 27          | 21/40        | —47       | 0.203/0.311 | —35       |
| " "            | II       | 0.5            | 32          | 22/39        | —44       | 0.266/0.311 | —15       |
| Mouse pn.      | I        | 0.4            | 27          | 11/25        | —56       | 0.161/0.209 | —23       |
| Meningo-pneum. | I        | 0.2            | 16          | 30/35        | —15       | 0.297/0.325 | — 8       |

\* T = Treated mice. C = Control mice.

† % change = 100(—1+T/C).

TABLE II.  
Effect of Arsenamide on Allantoic Infections in Chick Embryos.

| Virus               | Drug* time After virus, hr | Degree of infection as indicated by subinoculation of allantoic fluids into mice† |         |       |          |
|---------------------|----------------------------|-----------------------------------------------------------------------------------|---------|-------|----------|
|                     |                            | LS<20                                                                             | LS20-50 | LS>50 | Av. L.S. |
| Cat pneumonitis     | 1                          | 12                                                                                | 0       | 0     | 3.0      |
|                     | Sal.                       | 2                                                                                 | 5       | 7     | 52.0     |
|                     | 24                         | 7                                                                                 | 5       | 0     | 16.3     |
|                     | 48                         | 15                                                                                | 1       | 1     | 15.2     |
|                     | Sal.                       | 3                                                                                 | 1       | 4     | 46.0     |
| Meningo-pneumonitis | 1                          | 10                                                                                | 5       | 2     | 22.0     |
|                     | Sal.                       | 0                                                                                 | 1       | 11    | 87.0     |

\* Dose 0.2 mg/egg by allantoic route.

† LS<20, allantoic fluid not significantly infected; LS20-50, moderate infection; LS>50, heavily infected allantoic fluid.

dithioglycollate have a definite inhibitory activity against the viruses of mouse pneumonitis and cat pneumonitis in the lungs of mice. The dithioglycollate also inhibits

growth of the agents of meningopneumonitis and cat pneumonitis in the allantoic sac of chick embryos.

Received June 21, 1949. P.S.E.B.M., 1949, 71.

## 17240. Vitamin B<sub>12</sub> Content of Various Organs and Tissues of the Rat.\*

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The glandular tissues of various domestic animals have long been known to be effective in pernicious anemia when administered orally.<sup>1</sup> Beef liver and kidney have proved to be

the richest source of the active principle, kidney, however, possessing only one-half to two-

\* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

We are indebted to Merek and Company, Inc., Rahway, N. J., for crystalline vitamins and the crude B<sub>12</sub> concentrate, and to Dr. B. L. Hutchings of the Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y., for synthetic folic acid.

<sup>1</sup> Subbarow, Y., Hastings, A. B., and Elkin, M., *Vitamins and Hormones*, 1945, 3, 237.

TABLE I.  
Vitamin B<sub>12</sub> Content of Rat Tissues.

| Daily supplement                 | No. of animals per assay group | Series 1<br>Total avg gain (g)<br>2 wk | Series 2<br>Total avg gain (g)<br>2 wk | Min. B <sub>12</sub> (γ)<br>per g of sample |
|----------------------------------|--------------------------------|----------------------------------------|----------------------------------------|---------------------------------------------|
| None                             | 5                              | 48                                     | 37                                     | —                                           |
| .1 γ crystalline B <sub>12</sub> | 5                              | 73                                     | 66                                     |                                             |
| 1 g kidney                       | 3                              | 65                                     | 51                                     | 0.04                                        |
| 1 g " (B <sub>12</sub> )*        | 3                              | 89                                     | 87                                     | 0.17                                        |
| 1 g liver                        | 5                              | 47                                     | 31                                     | †                                           |
| 1 g " (B <sub>12</sub> )         | 5                              | 75                                     | 66                                     | 0.08                                        |
| 1 g intestine                    | 5                              | 38                                     | —                                      | †                                           |
| 1 g " (B <sub>12</sub> )         | 5                              | 62                                     | —                                      | 0.04                                        |
| 1 g heart                        | 1                              | —                                      | 36                                     | †                                           |
| 1 g " (B <sub>12</sub> )         | 1                              | —                                      | 62                                     | 0.07                                        |
| 1 g spleen                       | 1                              | —                                      | 32                                     | †                                           |
| 1 g " (B <sub>12</sub> )         | 1                              | —                                      | 30                                     | †                                           |
| 2.5 g muscle                     | 5                              | 57                                     | 43                                     | †                                           |
| 2.5 g " (B <sub>12</sub> )       | 5                              | 71                                     | 66                                     | Trace                                       |

\* (B<sub>12</sub>) From rats that received B<sub>12</sub> concentrate.

† No measurable quantity.

thirds the activity of liver. Spleen, brain, heart, and pancreas are effective to a lesser extent. These same organs from the pig and lamb have likewise been used beneficially in the dietary treatment of pernicious anemia.

An assay method has been reported<sup>2</sup> which can be used to measure fairly quantitatively the activity of anti-pernicious anemia preparations, and all subsequent work with the assay has indicated that vitamin B<sub>12</sub> is the active component being determined. The quantitative response obtained with crystalline vitamin B<sub>12</sub> affords a basis for the estimation of the amount of B<sub>12</sub> present in the material tested. Application of this was made in the testing of various beef and pork samples for B<sub>12</sub> content.<sup>3</sup> The work to be reported was undertaken to determine the relative concentration of vitamin B<sub>12</sub> in various organs and tissues of the rat in an effort to locate the main storage site of the vitamin within the body.

**Experimental. Animals.** The rats used as sources of the organ and tissue samples were obtained as weanlings. They were housed in stock colony cages and given food and water

*ad libitum*. A total of 30 rats were used and all received the corn-soybean ration described previously.<sup>4</sup> Fifteen of the animals were given in addition to this diet a vitamin B<sub>12</sub> concentrate (Merck and Co.) which was mixed directly into the basal ration to provide 3 γ of B<sub>12</sub> per 100 g of food. The rats were kept on these diets for 6 weeks before sacrificing.

**Preparation of samples.** The rats were decapitated, bled, and the tissues and organs removed. The liver, kidney, heart, spleen, small intestine, and the femoral muscles were taken. The livers, kidneys, and intestines were weighed, combined with an equal weight of distilled water, and homogenized in a Waring blender. The skeletal muscle was treated in the same manner except that a 3 to 1 dilution was necessary to obtain a smooth homogenate. All samples were stored at ordinary refrigeration temperature.

**Assay.** The assay method used has been described in a previous paper.<sup>2</sup> It consists of placing weanling rats on a basal ration for a 2-week depletion period and following the growth response during another 2-week period when the material to be tested is given. The samples were administered on alternate days in separate food containers. Four ml of the liver and kidney homogenates were given

<sup>2</sup> Register, U. D., Ruegamer, W. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1949, **177**, 129.

<sup>3</sup> Register, U. D., Lewis, U. J., Thompson, H. T., and Elvehjem, C. A., *Proc. Soc. Exp. Biol. and Med.*, 1949, **70**, 167.

<sup>4</sup> Jaffé, W. G., and Elvehjem, C. A., *J. Biol. Chem.*, 1947, **169**, 287.

every other day, thus supplying the animals with an equivalent of 1 g of the sample per day. Because of the higher dilution of the muscle homogenate and its probable lower B<sub>12</sub> content, 15 ml were administered which provided 2.5 g of muscle tissue per day.

The number of rats used for each assay group is recorded in Table I. A smaller number of rats was necessary in the cases of the kidney, spleen and heart because of an insufficient amount of sample. The assay was run in two series, a different group of rats being used as a source of the organs and tissues in each case.

*Results and discussion.* The data on the vitamin B<sub>12</sub> content of all the rat tissues and organs tested are presented in Table I. The values obtained for the heart and spleen are based on results from one assay animal and, therefore, are to be regarded only as indications of the actual.

The greater weight gain in the first series can be explained by the fact that the basal gain, that is, without supplement, was higher throughout all the groups. However, the relative values for B<sub>12</sub> content of the samples in the two individual series were similar.

The data show that in all instances, except possibly with the spleen, the inclusion of the B<sub>12</sub> concentrate in the animal's diet caused an

increase in the quantity of B<sub>12</sub> in the organs and tissues.

The kidneys were found to be the site of greatest B<sub>12</sub> concentration. Also the results indicate that the vitamin is retained in larger quantity for a longer period of time in the kidneys than in the other organs. The liver, heart, and intestine contained no appreciable amount of vitamin B<sub>12</sub> after the animals had been kept on the corn-soybean ration for 6 weeks. However, the increase was very marked in these organs, as with the kidney, upon the addition of the B<sub>12</sub> concentrate to the rat's diet. The skeletal muscle was not only lowest in B<sub>12</sub> but also remained most nearly constant in content.

*Summary.* The kidney, liver, heart, small intestine and femoral muscles of rats raised on a corn-soybean diet were assayed for vitamin B<sub>12</sub> content.

The kidney was found to be the site of greatest B<sub>12</sub> concentration. The liver, heart, small intestine, and muscle contained no appreciable amount of the vitamin after the animals had been kept on the basal ration for 6 weeks. The amount of B<sub>12</sub> increased in the organs and tissues when vitamin B<sub>12</sub> was added to the diet.

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Received June 22, 1949. P.S.E.B.M., 1949, 71.

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